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The tadpoles of the brown frogs *Rana [graeca] graeca* and *Rana [graeca] italica* (Amphibia, Anura)

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External morphology and buccopharyngeal characters of the tadpoles of *Rana [graeca] graeca* and *Rana [graeca] italica* are described in detail. Both characterize the larvae as well adapted to flowing waters. The most distinctive oral and buccal features are the increased number of tooth rows, the high number of papillae in both buccal floor and buccal roof arena, and the large prelingual palps with elongate lobes.

Larvae from Italy resemble those from Greece. However, samples from both countries differ slightly but significantly in a variety of features. This supports the existence of two taxons, subspecies or species, *graeca* for the populations of the Balkans and *italica* for those of the Apennines.

INTRODUCTION

Rana graeca Boulenger, 1891 (Greek frog, stream frog) is the only European anuran which was first recognized by its larva. The story of the tadpoles' discovery by the French batrachologist Louis-François HÉRON-ROYER is reported by BOULENGER (1891a) and confirmed by a letter from HÉRON-ROYER to Raymond ROLLINAT, dated 27 September 1891 (library, Laboratoire des Reptiles et Amphibiens, Muséum national d'Histoire naturelle, Paris), where he writes: "Je viens de recevoir une lettre de Boulenger qui

m'annonce une nouvelle grenouille pour la faune Européenne, sur un têtard de Grèce que je lui ai déterminé n'étant ni *R. fusca* ni *R. Latastei*. Il vient d'en faire une *Rana graëca*."

Geographical disjunction (map in Arnold & Burton, 1978) and morphometric divergences between adult specimens from the Balkans and from the Apennines gave rise to discussions on intraspecific variation (Arnold & Burton, 1978) and taxonomic status (Lanza, 1983). The establishment by Dubois (1987)[1] of two subspecies, *R. graeca graeca* (Balkans) and *R. graeca italica* (Apennines), was based on external morphometric differences in adults, whereas the suggested raising of *italica* to specific rank (Picariello et al., 1990; Capula, 1991) resulted from allozyme studies.

Literature on larval morphology of *R. graeca* is scarce. Oral disks are depicted in Boulenger (1891b) and Günther (1985), well developed larvae in Boulenger (1891b) and Beskov (1970). The former additionally provided a short description and differential diagnosis and the latter contributed to the knowledge of the tadpole's biology. Both authors refer to a small number of Balkan specimens only. No further morphological investigations are available and there are no comparative data on tadpoles from Italy.

The primary goal of the present paper is to describe the external and buccopharyngeal characters of *graeca* and *italica* larvae in the process of development. This is done for a variety of features (also for those where no significant differences between tadpoles from the Balkans and the Apennines were found), to make data available for comparison with other South European brown frog species.

Material and methods

Specimens from five Greek and seven Italian localities were investigated (Table I). Number of specimens is 212 for detailed morphometric analysis, 364 for size-stage diagram (fig. 4), and 282 for tooth rows counts. Description of buccopharyngeal structures refers to five tadpoles each (stages 36-38) from both Italy and Greece (asterisks in Table I) and is based on stereomicroscopy (n = 6) and scanning electron microscopy (n = 4).

External morphology is described using established parameters introduced by Boulenger (1897-1898), and defined in more detail by Grillitsch (1984) and Grillitsch et al. (1989). The measurements do not represent true distances but projections to the tadpole's frontal and sagittal planes respectively (Table II). Distances between pupillae or nostrils mean distances between the centres of these organs. Tooth rows of both upper and lower lip are numbered from the margin towards the centre of the oral disk, as is done in the classic terminology of Boulenger (1891b). The length of a tooth row is defined as the straight distance between its ends in the expanded oral disk. For tooth rows formula (number of upper rows / number of lower rows), rows are counted as one whether continuous or interrupted, uni- or bilateral.

1. Several authors (Picariello et al., 1990; Capula, 1991; Duellman, 1993) credit the name *italica* to "Dubois (1985)", although the paper where this name first appeared was published on 26 January 1987 (Dubois, 1988a), and should therefore be quoted as "Dubois (1987)".

Table I. — Material investigated. MNHN: Muséum national d'Histoire naturelle, Paris; NMW: Naturhistorisches Museum Wien; *: samples used for buccopharyngeal analysis; habitat: B, brook; T, torrent; R, river; P, pond; morphometry: specimens used for detailed morphometric analysis (Table III); size-stage graph: specimens used in size-stage graph (fig. 4); tooth rows counts: specimens used in tooth rows counts; n: number of specimens; RS: range of GOSNER's (1960) stages of specimens.

Specimens series	Country and region	Locality	Habitat	Date	Morphometry n (RS)	Size-stage graph n (RS)	Tooth rows counts n (RS)
MNHN 1985.1777-1815	Greece, Peloponnissos, Ahaia	Kato Vlassia (760-770 m)	T	13.08.82	34 (28-39)	36 (28-40)	34 (28-40)
NMW 29181:1-20	Greece, Peloponnissos, Ahaia	Krathis potamos near Zivlos (550 m)	B	06-10.08.84	12 (31-39)	15 (31-43)	14 (31-41)
MNHN 1985.1817-2024 *	Greece, Peloponnissos, Arkadia	Kalomeri (980 m)	T	14.08.82	66 (29-39)	182 (29-45)	117 (29-41)
NMW 27637:1-20	Greece, Peloponnissos, Ilia	Oros Minthi near Nea Figalia (750 m)	B	16.08.83	15 (27-39)	26 (27-41)	24 (27-41)
NMW 29180:1-20	Greece, Peloponnissos, Korinthia	Olvios potamos near Feneos (800 m)	B	06-10.08.84	10 (28-39)	10 (28-41)	10 (28-41)
MNHN 1985.1756-1775	Italy, Abruzzo, Teramo	Fiume Salinello (1040 m)	T	08.08.85	3 (34-39)	20 (34-44)	12 (34-41)
MNHN 1985.1719	Italy, Basilicata, Potenza	Fontana d'Eboli (1010 m)	B	02.08.82	1 (30)	1 (30)	1 (30)
MNHN 1985.1720	Italy, Basilicata, Potenza	Pecorone (800 m)	T	02.08.82	1 (37)	1 (37)	1 (37)
MNHN 1985.1504-1560 *	Italy, Calabria, Cosenza	Cosentino (1140 m)	T	22-23.07.82	57 (28-39)	57 (28-39)	55 (28-39)
MNHN 1985.1564-1674	Italy, Calabria, Cosenza	Fiume Savuto (1070 m)	R + P	24.07.82	1 (37)	1 (37)	-
MNHN 1985.1678-1691	Italy, Lazio, Frosinone	Vallegrande (530-570 m)	T	01.08.82	12 (37-39)	14 (37-40)	14 (37-40)
MNHN 1985.1776	Italy, Marche, Ascoli Piceno	Trisungo (630 m)	B	09.08.85	-	1 (42)	-

Table II. — Definition of distances measured, including explanation of abbreviations used. P: projection to frontal (F) or sagittal (S) plane.

Abbreviation	Definition	P
HT	Maximum height of tail (including upper and lower tail fin)	S
IMP	Number of inframarginal oral papillae	
LF	Maximum height of lower (ventral) tail fin	S
LTR1	Length of first (outermost) tooth row of lower lip	
LTR2	Length of second tooth row of lower lip	
MP	Number of marginal oral papillae	
NN	Internarial distance	F
NP	Naro-pupillar distance	F
OD	Maximum width of oral disk	F
PP	Interpupillar distance	F
RN	Rostro-narial distance	F
SS	Distance: tip of snout - opening of spiracle	S
SU	Distance: tip of snout - insertion of dorsal tail fin	S
SV	Distance: tip of snout - vent (snout-vent length)	S
TL	Distance: tip of snout - tip of tail (total length)	S
UF	Maximum height of upper (dorsal) tail fin	S
UTR1	Length of first (outermost) tooth row of upper lip	
UTR2I	Length of median gap between portions of second tooth row of upper lip	F
UTR2P	Length of one portion of second tooth row of upper lip	F
VS	Distance: vent - opening of spiracle	S
VT	Distance: vent - tip of tail (length of tail)	S

Nomenclature of buccopharyngeal structures is largely in accordance with Wassersug (1976, 1980); definition of developmental stages follows Gosner (1960).

Tadpoles examined comprise developmental stages 27 through 45; detailed morphometric analysis was restricted to stages 28-39. Since body proportions change during growth, morphometric data have to be accompanied by the size or developmental stage they refer to. In the present paper the assignment to size classes was preferred because of statistical reasons. Since there is a fair positive linear correlation between size and developmental stages 27 through 39 (fig. 4), they are easily convertible.

Measurements were done optically with a digital display length-measuring unit (Wild MMS 235). Preparation for SEM examination (Jeol JSM-35 CF) comprised dehydration (ethanol), critical-point-drying (acetone, liquid carbondioxide), and gold sputter surface-coating.

Statistical analyses were processed using SPSS-X and SAS. Significances (α) were calculated by means of Student t test and Mann-Whitney U test. Selection rule for discriminant analysis (fig. 10) with stepwise variable selection was: maximize minimum Mahalanobis distance. For both the pooled Greek and the pooled Italian samples, homogeneity was proved by Kruskal-Wallis ANOVA for each measurement within each of the six size classes, where sufficient material was available. For references concerning Haldane's coefficient of variation (Table III), see DELAUGERRE & DUBOIS (1985).

RESULTS

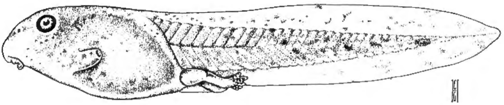
GENERAL APPEARANCE, COLOUR AND PATTERN (PRESERVED SPECIMENS) (FIGS. 1-3)

In Greek and Italian tadpoles, the slightly depressed ovoid body continuously extends into the robust, fairly elongate tail which lacks a marked constriction at its base. Both dorsal and ventral caudal fins are low and slightly convex with almost parallel edges. The height of the trunk is not or not clearly exceeded by that of the tail fin which is more or less tapering but never acutely pointed and sometimes even obtuse. As is typical of tadpoles of the subgenus *Rana*, the spiracular tube is sinistral and directed backwards and slightly upwards. It opens about halfway between tip of snout and vent, more frequently a little closer to the anterior than to the posterior end of the trunk, especially in advanced developmental stages. The vent opens subdextral, close to the edge of the ventral fin. The eyes are moderately sized, close to one another, not visible from below.

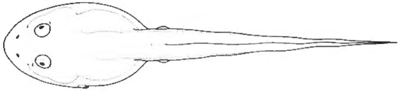
The trunk is dark greyish-brown above due to a close speckling with black. The ventral parts and the muscular portion of the tail are much lighter, the latter speckled with black. Caudal fins are greyish, transparent, with small dark spots or arborescent markings, mainly in the dorsal portion. There are neither distinct changes in colour or pattern during larval development, nor are there differences between Greek and Italian specimens.

SIZE AND PROPORTIONS OF TRUNK AND TAIL (TABLE III)

The tadpoles on which this study is based were all collected in the months of July and August (Table I), i.e. several months after the breeding period, which occurs in February to April in Italy (BAGNOLI, 1985; PICARIELLO et al., 1993) as well as in the Balkans (BESKOV, 1970; NÖLLERT & NÖLLERT, 1992). Total lengths (TL) of the smallest tadpoles examined were 20.2 mm (Italy, stage 28) and 21.5 mm (Greece, stage 28). So we cannot contribute to the size of hatchlings which is 9.1-9.5 mm for Bulgarian specimens (BESKOV, 1970). Maximum TL were 48.5 mm (Italy, stage 41) and 58.2 mm (Greece, stage 41), exceeding the maxima compiled from literature (45 mm, GÜNTHER, 1985; 46.3 mm,



1



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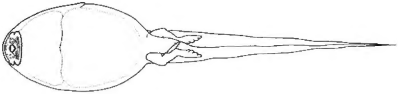

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Figs. 1-3. — Stage 38 *graeca* tadpole from Krathis potamos, Greece (NMW 29181): (1) lateral view; (2) dorsal view; (3) ventral view.

BESKOV, 1970; 48 mm, BOULENGER, 1891a-b; 50 mm, BAGNOLI, 1985). As in adults, the average TL of Greek larvae clearly surpasses that of Italian ones (fig. 4), what is significant ($\alpha \leq 0.05$) in stages 29, 31, 35, 36, 39, 40, 41.

Mean values of VT/SV varied with TL increasing from 0.78 to 1.53 in Italian, and from 0.92 to 1.45 in Greek larvae, exceeding 0.6 calculated from BOULENGER's (1891a) table. In size classes TL 30.0-49.99 mm, Italian tadpoles have longer tails than Greek ones ($\alpha \leq 0.05$).

The dorsal tail fin barely reaches the trunk. In Italian tadpoles, the dorsal fin generally extends a little more towards the trunk, whereas in Greek specimens it is restricted to the tail region. This difference in ratio SV/SU is significant ($\alpha \leq 0.01$) in size classes TL 35.0-44.99 mm.

Older (longer) larvae have relatively lower tail fins. The means of VT/HT vary from 1.46 (young larvae) to 2.88 (advanced stages) in Italian tadpoles, and from 2.26 to 3.81 in Greek specimens, respectively, indicating conspicuously higher fins in Italian larvae. These differences are significant ($\alpha \leq 0.05$) in specimens longer than TL 25.0 mm.

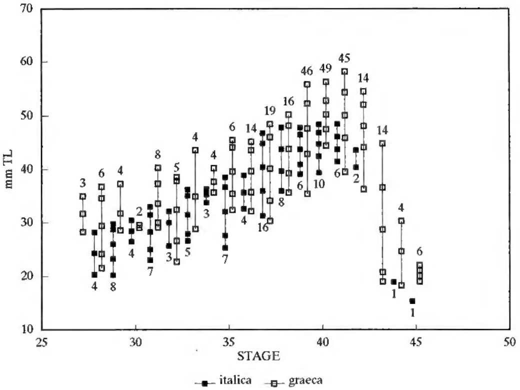

Fig. 4. — Size-stage graph, showing correlation of size (TL) and developmental stage in *italica* and *graeca* tadpoles, including mean value, range, standard deviation and sample size.

On the average, in Italian individuals the heights of dorsal and ventral tail fins are almost the same (UF/LF around 1.0). In Greek tadpoles, the dorsal fin is usually higher than the ventral one (means of UF/LF 1.11-1.34). Differences are significant ($\alpha \leq 0.01$) in animals longer than TL 30 mm.

Ratios HT/UF and SS/VS reveal no significant differences between Italian and Greek tadpoles.

POSITION OF EYES AND NARES, WIDTH OF ORAL DISK

The nares are positioned closer to the tip of the snout than to the eyes. Mean values of RN/NP are a little higher in Greek than in Italian larvae, meaning the nares of the Italian being closer to the tip of the snout ($\alpha \leq 0.1$ in size classes TL 30.0-34.99 mm and TL 40.0-49.99 mm). Ratio PP/NN is not significantly different between Italian and Greek larvae.

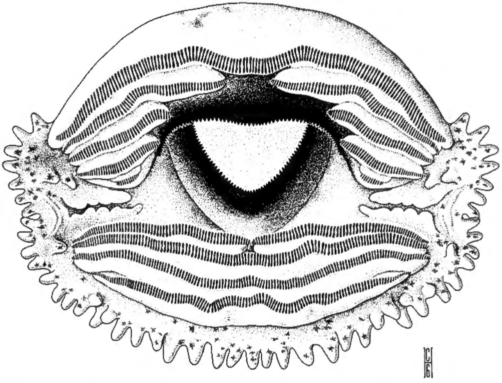

Fig. 5. — Oral disk of a *graeca* tadpole (stage 38) from Krathis potamos, Greece (NMW 29181), stage 38.

Boulenger (1891a-b) mentions that *R. graeca* tadpoles differ from their European "congeners in having the mouth quite as wide as the interorbital space". Mean values of PP/OD vary from 1.05 to 1.18. Greek tadpoles show a comparatively wider oral disk ($\alpha \leq 0.05$) in size classes TL > 40 mm.

Oral disk (figs. 1, 3, 5, 6)

The *oral disk* is in ventral subterminal position. It is expanded laterally and of ovoid or rectangular shape. Marginal peribuccal papillae (MP) are restricted to the lateral corners and the posterior margin of the oral disk, and are basically arranged in a single row at a density of 9-10 per millimetre on the posterior margin. In the lateral corners, besides solitary inframarginal papillae (IMP), two papillate ridges are descending towards the beak on each side (figs. 5, 6).

Table III. — Descriptive statistics of selected parameters describing *graeca* (G) and *italica* (I) larvae. Size classes are according to the value of TL (mm). n: number of specimens; min: minimum value; med: median value; $\bar{x}$: mean value; max: maximum value; Sx: standard error of the mean, Sd: standard deviation; V_H: HALDANE's coefficient of variation. For further abbreviations see Table II.

Size class	20.0-24.99		25.0-29.99		30.0-34.99		35.0-39.99		40.0-44.99		45.0-49.99		50-54.9	55-55.9
Samples	I	G	I	G	I	G	I	G	I	G	I	G	G	G
Stage														
n	6	2	19	12	15	16	14	34	12	23	8	38	11	2
min	28	28	28	27	30	27	33	27	37	31	37	35	38	37
med	29	30	30	29/30	34	35/36	36/37	36	37	37	38	39	39	38
max	31	32	35	33	37	37	39	39	39	39	39	39	39	39
TL														
n	6	2	19	12	15	16	14	34	12	23	8	38	11	2
min	20.20	21.50	25.10	26.10	30.30	30.00	35.10	35.00	40.80	40.20	45.20	45.10	50.20	55.40
x	22.43	22.10	27.34	28.58	32.56	32.31	37.25	37.25	42.91	42.81	46.78	48.07	51.56	55.60
max	24.20	22.70	29.80	29.60	34.50	34.50	39.10	39.80	44.50	44.90	47.90	49.90	54.60	55.80
Sx	0.73		0.34	0.28	0.31	0.38	0.39	0.26	0.39	0.32	0.36	0.22	0.42	
Sd	1.78		1.49	0.96	1.21	1.50	1.45	1.50	1.33	1.53	1.01	1.36	1.39	
V_H	8.3		5.5	3.5	3.8	4.7	4.0	4.1	3.2	3.6	2.2	2.9	2.8	
SV														
n	6	2	19	12	15	16	14	34	12	23	8	38	11	2
min	11.40	10.70	11.40	12.00	13.70	13.40	14.70	15.30	16.60	17.40	17.00	18.30	20.20	22.40
x	12.73	11.60	13.34	14.23	15.00	15.76	16.22	16.92	18.10	19.24	18.49	20.20	21.26	22.75
max	14.80	12.50	15.20	19.40	16.00	18.90	17.80	19.60	19.30	20.60	19.40	22.40	23.30	23.10
Sx	0.56		0.24	0.51	0.17	0.33	0.24	0.18	0.25	0.20	0.34	0.19	0.27	
Sd	1.38		1.04	1.78	0.67	1.30	0.90	1.06	0.87	0.95	0.95	1.17	0.91	
V_H	11.3		7.9	12.8	4.5	8.4	5.7	6.3	4.9	5.0	5.3	5.8	4.4	
VT/SV														
n	6	2	19	12	15	16	14	34	12	23	8	38	11	2
min	0.51	0.82	0.69	0.48	0.97	0.41	1.16	0.88	1.15	1.09	1.43	1.09	1.22	1.40
$\bar{x}$	0.78	0.92	1.06	1.03	1.17	1.00	1.30	1.21	1.38	1.23	1.53	1.39	1.43	1.45
max	1.10	1.01	1.42	1.36	1.41	1.38	1.51	1.44	1.68	1.43	1.71	1.64	1.58	1.49
Sx	0.09		0.05	0.06	0.03	0.07	0.03	0.02	0.04	0.02	0.04	0.02	0.03	
Sd	0.21		0.20	0.21	0.11	0.27	0.12	0.11	0.14	0.11	0.11	0.15	0.10	
V_H	25.40		18.20	19.80	9.60	26.40	9.40	9.20	10.40	9.00	6.70	10.90	6.40	
SV/SU														
n	6	2	18	11	14	16	14	34	12	23	8	33	10	2
min	1.13	0.99	1.05	1.01	0.85	0.99	0.98	0.97	1.08	1.05	1.06	0.91	1.04	1.06
$\bar{x}$	1.25	1.06	1.24	1.18	1.23	1.18	1.21	1.12	1.26	1.16	1.16	1.16	1.12	1.21
max	1.50	1.13	1.51	1.34	1.38	1.54	1.44	1.28	1.45	1.28	1.35	1.45	1.24	1.35
Sx	0.05		0.03	0.04	0.04	0.03	0.03	0.01	0.03	0.01	0.04	0.02	0.02	
Sd	0.13		0.14	0.12	0.14	0.14	0.13	0.07	0.11	0.07	0.10	0.10	0.06	
V_H	10.00		10.60	9.50	10.80	11.20	10.10	6.30	8.10	5.20	8.00	7.80	4.60	

SS/VS														
n	6	2	18	11	14	16	14	34	12	23	8	33	10	2
min	0.79	0.99	0.80	0.80	0.77	0.79	0.79	0.72	0.75	0.73	0.75	0.70	0.63	0.76
x	0.96	1.00	0.93	0.95	0.92	0.90	0.91	0.90	0.92	0.86	0.91	0.91	0.89	0.82
max	1.05	1.01	1.28	1.34	1.05	1.00	1.12	1.24	1.01	1.05	1.08	1.17	1.04	0.88
Sx	0.05		0.03	0.04	0.02	0.02	0.03	0.02	0.02	0.02	0.04	0.02	0.04	
Sd	0.11		0.10	0.14	0.08	0.06	0.10	0.11	0.08	0.09	0.12	0.09	0.12	
V_H	10.90		10.90	14.00	8.90	6.80	11.20	12.30	8.90	10.60	13.60	10.00	12.70	
VT/HT														
n	6	2	18	11	14	16	14	34	12	23	8	33	10	2
min	0.97	1.78	1.30	1.28	1.66	1.00	2.10	1.88	2.27	2.88	2.25	2.90	3.51	2.97
$\bar{x}$	1.46	2.26	2.09	2.44	2.24	2.47	2.52	3.22	2.55	3.35	2.88	3.54	3.81	3.31
max	1.89	2.73	2.83	3.31	2.78	3.58	3.39	3.91	2.99	3.92	3.19	4.23	4.26	3.64
Sx	0.15		0.11	0.15	0.07	0.17	0.10	0.08	0.07	0.07	0.11	0.06	0.08	
Sd	0.36		0.47	0.49	0.25	0.69	0.36	0.48	0.23	0.34	0.30	0.34	0.26	
V_H	23.50		21.80	19.40	10.10	27.10	14.10	14.70	8.80	10.00	10.00	9.40	6.70	
HT/UF														
n	6	2	17	11	14	16	13	34	12	23	8	33	10	2
min	3.03	3.09	2.90	2.98	2.91	2.66	2.76	2.74	2.69	2.99	2.84	2.95	2.92	3.61
$\bar{x}$	3.32	3.51	3.47	3.49	3.34	3.27	3.31	3.47	3.38	3.58	3.20	3.55	3.62	3.62
max	3.92	3.92	4.72	3.96	3.74	4.53	3.74	4.79	4.09	4.64	3.71	4.80	4.88	3.62
Sx	0.13		0.12	0.10	0.07	0.11	0.08	0.08	0.12	0.08	0.11	0.07	0.20	
Sd	0.31		0.51	0.32	0.26	0.46	0.30	0.47	0.41	0.40	0.31	0.39	0.64	
V_H	8.80		14.60	8.80	7.60	13.70	8.90	13.40	12.10	11.00	9.30	10.80	17.30	
UF/LF														
n	6	2	17	11	14	16	13	33	12	23	8	32	10	2
min	0.79	1.16	0.62	0.82	0.83	0.78	0.90	0.83	0.82	0.88	0.01	0.01	0.90	1.09
$\bar{x}$	0.98	1.34	0.97	1.11	1.00	1.25	1.10	1.26	1.03	1.24	0.99	1.26	1.34	1.10
max	1.10	1.52	1.34	1.32	1.23	1.63	1.58	1.73	1.26	1.60	1.23	1.73	1.83	1.11
Sx	0.05		0.04	0.05	0.03	0.06	0.06	0.04	0.04	0.04	0.14	0.05	0.08	
Sd	0.11		0.18	0.17	0.12	0.23	0.20	0.21	0.13	0.20	0.40	0.31	0.26	
V_H	10.60		19.00	14.90	11.20	18.70	21.10	16.80	11.90	15.50	7.40	15.60	18.40	
PP/NN														
n	6	2	18	11	14	14	14	33	12	23	8	33	10	2
min	1.43	1.49	1.22	1.29	1.33	1.33	1.34	1.35	1.46	1.47	1.52	1.46	1.61	1.66
$\bar{x}$	1.50	1.50	1.41	1.45	1.50	1.53	1.64	1.58	1.60	1.58	1.66	1.67	1.74	1.67
max	1.69	1.51	1.68	1.55	1.69	1.74	2.12	2.10	1.79	1.72	1.81	1.95	1.85	1.68
Sx	0.04		0.02	0.03	0.03	0.02	0.07	0.03	0.03	0.01	0.04	0.02	0.02	
Sd	0.10		0.10	0.08	0.11	0.11	0.28	0.19	0.10	0.07	0.11	0.13	0.08	
V_H	6.30		6.50	5.70	6.80	7.30	16.80	12.10	5.80	3.80	6.20	7.20	4.10	
RN/NP														
n	6	2	18	11	14	16	14	34	12	23	8	33	10	2
min	0.49	0.64	0.45	0.48	0.50	0.56	0.47	0.46	0.37	0.45	0.46	0.47	0.42	0.58
x	0.66	0.65	0.59	0.63	0.61	0.69	0.67	0.68	0.57	0.65	0.55	0.63	0.61	0.59
max	0.88	0.65	0.75	0.86	0.81	0.87	0.94	0.85	0.72	0.81	0.70	0.93	0.68	0.60
Sx	0.06		0.02	0.03	0.02	0.02	0.04	0.02	0.03	0.02	0.03	0.02	0.03	
Sd	0.14		0.08	0.11	0.09	0.10	0.14	0.09	0.11	0.10	0.08	0.10	0.08	
V_H	20.50		12.00	16.20	13.30	13.20	19.80	13.30	17.90	15.60	13.10	14.40	13.40	

PP/OD														
n	6	2	17	11	14	16	14	33	12	23	8	33	10	1
min	1.01	1.05	0.95	0.86	0.91	0.85	0.95	0.88	1.12	0.90	1.21	0.98	1.01	1.16
$\bar{x}$	1.12	1.14	1.08	1.05	1.13	1.14	1.15	1.18	1.26	1.18	1.34	1.11	1.15	
max	1.24	1.23	1.20	1.21	1.30	1.40	1.32	1.36	1.45	1.40	1.45	1.26	1.26	1.16
Sx	0.04		0.02	0.03	0.03	0.04	0.03	0.02	0.03	0.03	0.03	0.02	0.02	
Sd	0.10		0.08	0.11	0.11	0.14	0.12	0.12	0.10	0.15	0.10	0.08	0.07	
V_H	8.40		6.60	10.70	9.90	11.60	9.70	10.30	8.10	12.00	6.90	7.30	6.20	
NN/OD														
n	6	2	17	11	14	14	14	34	12	23	8	33	10	1
min	0.68	0.70	0.68	0.62	0.63	0.61	0.46	0.51	0.72	0.56	0.71	0.54	0.60	0.69
$\bar{x}$	0.75	0.76	0.78	0.73	0.76	0.75	0.72	0.76	0.79	0.75	0.81	0.67	0.66	
max	0.83	0.82	0.89	0.82	0.87	0.87	0.84	0.92	0.84	0.90	0.89	0.80	0.73	0.69
Sx	0.02		0.01	0.02	0.02	0.02	0.03	0.02	0.01	0.02	0.02	0.01	0.01	
Sd	0.06		0.05	0.07	0.08	0.08	0.12	0.10	0.04	0.10	0.06	0.07	0.04	
V_H	7.00		6.50	9.80	10.70	9.50	15.60	13.30	5.20	12.10	7.60	9.00	4.70	
UTR2P/UTR2I														
n	6	2	17	10	13	16	13	34	8	22	6	32	9	2
min	3.50	4.18	2.07	3.12	2.24	2.20	3.62	2.64	4.76	2.95	5.48	1.00	3.88	4.69
$\bar{x}$	5.16	4.43	6.87	6.36	9.05	6.05	6.22	7.19	8.15	8.25	11.89	8.90	10.69	7.70
max	7.70	4.68	26.40	10.59	22.57	12.67	10.36	37.33	13.45	22.33	20.00	51.00	46.25	10.70
Sx	0.69		1.46	0.89	1.83	0.91	0.61	1.10	1.02	1.06	2.44	1.62	4.46	
Sd	1.68		6.02	2.81	6.58	3.63	2.21	6.39	2.87	4.95	5.99	9.15	13.39	
V_H	33.9		88.9	45.3	74.1	60.9	36.2	89.5	36.3	60.7	52.5	103.6	128.7	
LTR2/LTR1														
n	6	2	17	9	13	15	14	33	11	22	8	33	10	2
min	1.03	1.09	0.87	0.83	0.68	1.00	0.65	0.83	1.02	0.61	1.05	0.86	1.01	1.08
$\bar{x}$	1.10	1.17	1.10	1.11	1.07	1.12	1.07	1.12	1.08	1.11	1.12	1.12	1.11	1.22
max	1.15	1.25	1.25	1.34	1.30	1.22	1.38	1.27	1.16	1.41	1.22	1.50	1.27	1.36
Sx	0.02		0.02	0.05	0.04	0.02	0.05	0.02	0.01	0.03	0.02	0.02	0.03	
Sd	0.05		0.08	0.14	0.14	0.06	0.20	0.09	0.05	0.15	0.06	0.11	0.09	
V_H	3.80		7.40	13.00	12.40	5.50	18.10	8.10	4.70	12.80	5.50	9.00	8.30	
IMP														
n	6	2	17	11	13	16	14	32	12	23	8	33	10	2
min	10	1	6	2	9	1	7	3	5	1	5	1	2	7
x	14.00	5.00	15.53	6.82	16.31	9.75	14.71	7.88	13.17	6.74	13.75	6.09	6.50	8.00
max	22	9	36	12	23	16	21	17	24	11	27	15	15	9
Sx	1.70		1.74	0.87	1.18	0.96	0.98	0.58	1.75	0.54	2.47	0.59	1.18	
Sd	4.16		7.19	2.89	4.27	3.83	3.67	3.26	6.05	2.61	6.98	3.39	3.75	
med	13	5	14	7	18	10	15/16	7	11/12	7	13	5	5/6	8
V_H	31.00		47.00	43.30	26.70	39.90	25.40	41.70	46.90	39.10	52.40	56.10	58.70	
MP														
n	6	2	17	11	13	16	14	32	12	23	8	33	10	2
min	59	43	55	51	64	57	53	57	66	60	65	67	80	61
x	66.83	54.00	74.06	69.00	79.85	71.19	80.43	71.94	79.17	76.26	80.75	91.97	93.20	75.50
max	75	65	99	84	92	87	100	95	89	99	102	114	118	90
Sx	2.30		2.60	2.61	2.39	1.72	3.24	1.34	2.29	2.26	4.37	2.15	4.24	
Sd	5.64		10.72	8.64	8.62	6.86	12.13	8.10	7.95	10.82	12.37	12.36	13.64	
med	66/67	54	74	69	84	72/73	79/80	72	81	75	79/80	92	88	75/76
V_H	8.80		14.70	12.80	11.00	9.80	15.40	11.40	10.30	14.30	15.80	13.50	15.10	

Fig. 6. SEM micrograph of left corner of the oral disk of a *graeca* tadpole (stage 38) from Krathis potamos, Greece (NMW 29181)

Through all size classes up to TL ≤ 55 mm, mean numbers of MP increase constantly from 54 to 93 in Greek, and from 67 to 81 in Italian larvae. There are always significant differences ($\alpha \leq 0.1$) between Italian and Greek specimens. However, in size classes TL < 45 mm, Italian larvae have more papillae than Greek ones, while in longer larvae the contrary is observed (Table III).

Inframarginal papillae (IMP) are frequently found in the corners of the mouth or solitary inside the marginal papillae. Their number is significantly ($\alpha \leq 0.01$) higher in Italian than in Greek tadpoles of TL ≥ 30 mm (Table III).

In tadpoles at stages 27 through 41, there are usually 4-5 rows of keratodonts (*tooth rows*) in the anterior and 4 in the posterior lip. Keratodonts are disposed in single series on each ridge. In all tooth rows of tadpoles at stages 36-38, density of keratodonts is 7-8 per 100 µm; they are 70-80 µm long and their apical portions are spatulate with 12-14 acute marginal denticles each (fig. 7).

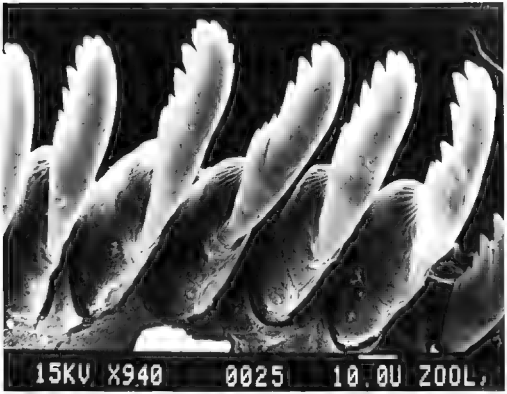

Fig. 7 SEM micrograph of keratodonts of a *graeca* tadpole (stage 38) from Krathis potamos, Greece (NMW 29181)

The outermost upper row (UTR1) and the outer three lower rows (LTR1-3) are continuous and almost equal in length. The innermost lower row (LTR4) reveals a short median gap without exception in our specimens; however, according to BOULENGER (1891a-b), it may also be continuous. Width of median gap is wide in UTR3-5, and moderate to short in UTR2 (Table III). Ratios LTR2/LTR1 and UTR2P/UTR2I in Italian and Greek larvae do not differ significantly.

Both lateral extension of upper tooth rows and length of their left and right portion decrease in centripetal direction; the portions of the innermost extremely short row (UTR5) bear a few keratodonts only, and may be unilateral or even absent. Absence is more frequent in, but not restricted to, early developmental stages.

In both Italian and Greek tadpoles, the total number of tooth rows slightly increases during development. Two tooth row formulae were found frequently: 4/4 (in 30 specimens of *italica* and 29 of *graeca*) and 5/4 (in 53 and 164 specimens, respectively). Two much rarer formulae were observed exclusively in *graeca*: one specimen (stage 39) unilaterally showed a distinct innermost UTR6 (formula 6/4); in five specimens (stages 29, 33, 39, 40,

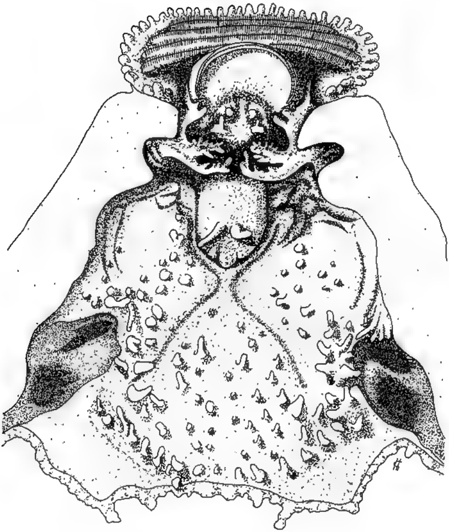

Fig 8 — Buccal floor of a *graeca* tadpole (stage 38) from Nea Figalia, Greece (NMW 27637).

41), a short outermost, fifth LTR, one fourth to one tenth of the length of LTR1, was present in a median position (formula 5/5).

The *jaw sheaths* (beak) are robust with dark pigmentation, the upper cutting edge is gently "M"-shaped, the lower one "U"-shaped; there are about 5 serrations (45-50 µm high) per 100 µm in both sheaths of tadpoles of stages 36-38.

Fig. 9. - Buccal roof of a *graeca* tadpole (stage 38) from Nea Figalia, Greece (NMW 27637).

BUCCOPHARYNGEAL CAVITY

In the *buccal floor* (fig. 8), two pairs of stout, jointed ventral infrarostral pustulations form a semicircular arch within the median third of the prelingual area The pair of prelingual palps is large, with three slender, elongate, finely-limbed, and secondarily papillate lobes, long enough to reach out of the mouth.

Two slim cylindric lingual papillae rise in the posterior half of the distinct tongue anlage.

The buccal floor arena is scattered regularly with about 100 conical, elongate papillae, which are almost as long as the lingual papillae; there are few small pustulations in between. Prepocket papillae can be even larger and furcated or palp-like.

The margin of the velar apparatus describes a smooth, broad arch with three pairs of conical marginal projections corresponding to the filter cavities; the median portion of the velum is smooth-edged showing two further papilla-like projections on each side of the quite undistinct median notch. The glandular zone is broad, not markedly thickened, with distinct secretory pits, glottis and laryngeal disk are not exposed.

In the *buccal roof* (fig. 9), the prenarial area shows three pairs of tuberous papillae, arranged in a semicircular arch; the most anterior pair is polydactylous. The main axis of the internal nares is almost in a right angle to the main body axis. In the centre of the anterior narial walls a slender, papillate flap is rising on each side; medially, the wall is lined with a few minor pustulations. The posterior walls of the nares are smooth-edged valves with a slight lobe towards the midline on each side. There is a single pair of slender, elongate postnarial papillae, with a line of pustulations on the anterior margin, and only one pair of small, cylindric lateral ridge papillae with two or three terminal pustulations which may be accompanied by two tiny pustules each. The median ridge is forming an almost isogonic triangular flap; its lateral margins are bordered by three or four pustulations.

The high number of about 70 buccal roof arena papillae corresponds to that in the buccal floor, but the dorsal ones are markedly shorter. The dorsal velum is well developed, showing a broad zone with distinct secretory pits.

No obvious differences were found between Italian and Greek tadpoles.

Discussion and conclusions

Morphological adaptations to life in flowing waters

Most of the tadpoles of *graeca* and *italica* on which this study is based were collected in flowing waters. small brooks, torrents of various sizes or larger rivers (Table I). Only one series of *italica* was collected in part in a river (Fiume Savuto) and in part in a pond in the bed of this river and close to the flowing river itself: probably the eggs were laid there before the pond was isolated from the river by the lowering of its level.

The larvae of *graeca* and *italica* are highly adapted to flowing waters by both external and buccopharyngeal characters. These comprise the slightly depressed body, the relatively long tail with low dorsal and ventral caudal fin, the former barely reaching the trunk, as well as the subterminal oral disk with the highest number of tooth rows among European ranine larvae.

In the lateral corners of the oral disk, besides the solitary inframarginal papillae, two papillate ridges are descending towards the basis of the beak on each side (figs. 5-6). In

this region, folds and additional papillae are often seen in anuran larvae, but distinct pairs of ridges have not been reported before; they might support the suctorial function of the oral disk in separating upper and lower "lips", and thus, possibly enable maintainance of low-pressure in the posterior portion while the anterior part may be loose. Though the importance of oral disk suction in flowing waters is evident, too little is known on mechanics of the peribuccal structures in feeding and adhesion for clear functional interpretation.

The pair of prelingual palps, long enough to reach out of the mouth, is a feature of the stream-adapted, bottom-feeding type as characterized by WASSERSUG (1980).

The number of buccal floor papillae (about 100) is at least twice that of *R. temporaria* given by VIERTEL (1982). In European ranine frogs, usually 40 to 60 papillae are found in this area, the lateral ones tending to be more elongate, the median ones often being low pustules. In general, these papillae are more numerous and elongate in stream-adapted larvae feeding on a self-generated suspension, and are serving as a coarse pre-filter (WASSERSUG, 1980). This also has been noted by GRADWELL (1972) for tadpoles of *Rana fuscigula* which live in "quiet pools in cool mountain streams".

The larvae of *graeca* and *italica* belong to the few lotic European tadpoles which also include the larva of the Majorcan midwife toad, *Alytes muletensis* (VIERTEL, 1984), the tadpoles of *R. iberica* and *R. pyrenaica* (SERRA-COBO, 1993), and possibly at least of some populations currently referred to *R. temporaria* and to the *R. macrocnemis* complex.

As concerns the ecomorphological guilds of exotrophic anuran larvae (ALTIG & JOHNSTON, 1989), *graeca* and *italica* have to be assigned to the lotic, rheophilous type, moderately expressing the characters of the "clasping" subtype

COMPARISON WITH OTHER EUROPEAN FROGS OF THE GENUS *RANA*

In four European brown frog species there is a tendency towards irregular development of the outermost lower tooth row and the innermost upper tooth row, concerning UTR5 in *graeca* and *italica* (present paper), UTR4 in *R. t. temporaria*, and UTR3 in *R. dalmatina* and *R. arvalis wolterstorffi* (GRILLITSCH & GRILLITSCH, 1989). Although early posthatching stages are not on hand, *graeca* and *italica* seem to fit into the general pattern of tooth rows development within the European brown frogs, which means: rows of keratodonts being additional to the basic formula of 2/3 show retarded ontogenetic appearance, are added centripetally in the upper, centrifugally in the lower lip, and reveal the more susceptibility to alterations the later they occur (GRILLITSCH & GRILLITSCH, 1989). Both retarded ontogenetic appearance and irregular formation suggest these additional tooth rows to be of young phylogenetic age

For differential diagnosis to sympatric *R. temporaria*, *R. dalmatina* and green frogs larvae, the tooth rows formulae of *graeca* and *italica* (4-5/4 in *italica*, 4-6/4-5 in *graeca*) appear to be the most suited and easy to handle external character It may fail in very young specimens (TL < 20.0 mm) and in advanced specimens with already reduced number of tooth rows, and then may lead to confusion, especially with *R. temporaria*.

In the samples studied, the following three buccopharyngeal characters of *graeca* and *italica* (stages 36-38) showed distinct differences compared to the other European brown frog tadpoles for which these characters were already described:

– In ranine frogs, two or four lingual papillae occur, the latter type being most common (Hammerman, 1964; Viertel, 1982; Inger, 1985). According to Viertel (1982), the number of lingual papillae is useful to separate European brown frogs (subgenus *Rana (Rana)* sensu Dubois, 1992) from European green frogs (subgenus *Rana (Pelophylax)* sensu Dubois, 1992), the former developing four, the latter two lingual papillae. Yet, *graeca* and *italica* tadpoles have two papillae, which contradicts the above classification.

– Comparing the total counts of velar marginal projections, Viertel (1982) gives them as 5-6 in European brown frogs and about 10 in European green frogs; *graeca* and *italica* with a number of 10 match the latter. This cancels the character for group clustering but separates *graeca* and *italica* from the other brown frogs.

– In *graeca* and *italica*, the longitudinal axis of the internal nares is almost in a right angle to the main body axis; this is different from all other European *Rana* species where the choanae form an anteriorly opened, obtusely angled "V" (Viertel, 1982).

All the characters mentioned above support the proposal of Dubois (1992) to recognize, within the subgenus *Rana (Rana)* s. str., a distinct species group (*Rana graeca* group) for *graeca* and *italica*.

The status of *graeca* and *italica*

Larvae from Italy and Greece could not be distinguished unequivocally from each other on the basis of their buccopharyngeal morphology. However, *graeca* has a significant tendency to have more tooth rows in the anterior lip than *italica*, especially in older stages. Besides, there are slight but significant differences between them in a variety of external morphometric features (SV(TL)/stage; ratios VT/HT, UF/LF, VT/SV, SV/SU, RN/NP, PP/OD, NN/OD; numbers of MP and IMP). Depending on developmental stage these differences are of variable diagnostic significance. "Coefficients of difference" (Géry, 1962; Mayr, 1975) were calculated for every metric character in all size classes. Out of 70 coefficients, 66 (i.e. 94 %) were low (between 0.0 and 0.71), indicating that thereby less than 70 % of the individuals can be assigned correctly to one of the groups, Italy or Greece. Only four coefficients (Table IV) came close to or even surpassed the usual conventional degree (1.28) of subspecific divergence, suggesting that, with their help, 85-92 % of the individuals can be assigned to the right group. The more the tadpoles develop, the more evident become the differences between Italian and Greek larvae. The mean coefficient of difference of all 14 proportions increases from 0.22 (TL 25.0-29.99 mm) to 0.49 (TL 45.0-49.99 mm).

Discriminant analyses executed for 6 size classes revealed two isolated clusters (Italy and Greece), to which 87-100 % of the individuals were assigned properly (fig. 10).

This study therefore demonstrates the existence of a significant morphological dissimilarity between the tadpoles of Italy and Greece. Addition of this third piece of

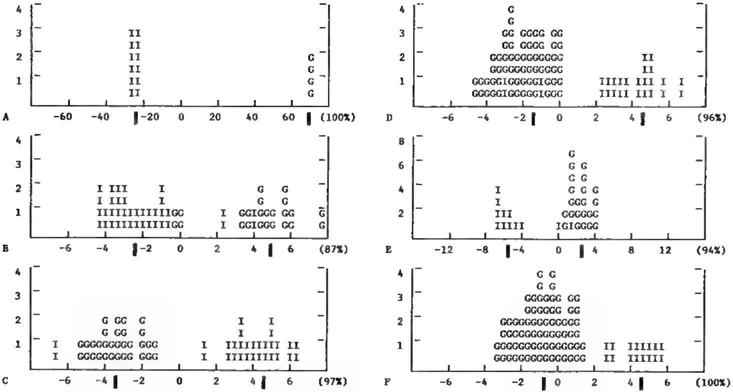

Fig. 10. — Six size classes (A: 20.0-24.99 mm, B. 25.0-29.99 mm; C: 30.0-34.99 mm; D: 35.0-39.99 mm, E: 40.0-44,99 mm, F. 45.0-49.99 mm) of 212 *graeca* (G) and *italica* (I) tadpoles clustered by discriminant analysis with stepwise variable selection. The percentage of proper assignement is indicated at the right end of the abscissa. Vertical bars (I) at the bottom symbolize class centroids. The ordinate represents the frequency (number of individuals), the abscissa shows canonical discriminant function scores.

Table IV. Ratios and size classes where coefficients of difference between *graeca* and *italica* are close to the usual conventional level of subspecific separation (1.28). For abbreviations see Table II.

Ratio	Size class	Coefficient of difference
VT/HT	40.00-44.99 mm	1.40
VT/HT	45.00-49.99 mm	1.03
PP/OD	45.00-49.99 mm	1 28
NN/OD	45.00-49.99 mm	1.08

evidence to the first two already available (adult morphology: DUBOIS, 1987; allozymes: PICARIELLO et al., 1990; CAPULA, 1991; GOLLMANN, 1992), confirms that both forms should be treated as different taxons Should they be considered subspecies or species? Since these forms are fully allopatric, not connected by a contact zone, this question is difficult to answer (see e.g. the detailed discussion in DUBOIS, 1977), and at this stage of research we prefer to keep this question open. We disagree with several current authors regarding the taxonomic weight and meaning of "molecular distances" (see DUBOIS, 1988b: 50, for a criticism of the use of the name "genetic distance" for such indices): these distances can be based on the results of electrophoreses (e.g. NEI's or ROGERS' distances), on immunological comparisons, or on nucleic acids hybridizations or direct comparison of their sequences. Contrary to what is believed by some current workers, including batrachologists (CEI, 1971; CRESPO, 1972; LANZA et al., 1975, 1976, 1982, 1984; BUSACK et al., 1985; CAPULA et al., 1985; BUSACK, 1986; etc.), a high "molecular distance" between two allopatric populations or groups of populations is not *by itself* sufficient evidence that they belong to distinct species: it can just be one piece of evidence among others, with no more weight than evidence from morphology, mating call, chromosomes, etc. As analysed in detail by PASTEUR & PASTEUR (1980) and PASTEUR (1985), there exists no such thing as a "specific level" of molecular differentiation· for example, two different good species may be separated by a "molecular distance" much weaker than that between populations of another species. Therefore, proper resolution of the status of *graeca* and *italica* will require additional work, dealing with other characters (e. g. hybridization, eco-ethology, mating calls, nucleic acids, chromosomes, etc.).

RÉSUMÉ

La morphologie externe et l'anatomie buccopharyngée des têtards de *Rana [graeca] graeca* et *Rana [graeca] italica* sont décrites en détail. Ces caractères traduisent une bonne adaptation de ces têtards à la vie en eau courante. Les particularités buccales les plus

notables sont le nombre élevé de rangées de kératodontes, le nombre élevé de papilles sur le plancher et le plafond buccal, et les grands palpes prélinguaux à lobes allongés.

Les têtards provenant d'Italie ressemblent beaucoup à ceux de Grèce. Toutefois, les deux groupes s'avèrent différer légèrement mais de manière significative l'un de l'autre pour un certain nombre de caractères. Ces résultats confirment l'existence de deux taxons distincts, sous-espèces ou espèces, *graeca* pour les populations des Balkans et *italica* pour celles des Apennins.

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Anurans of Borjomi Canyon: clutch parameters and guild structure

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Spawn clutches of six anuran species, inhabiting Borjomi Canyon in central Georgia, were examined: *Rana macrocnemis, Bufo [bufo] verrucosissimus, Pelodytes caucasicus, Rana ridibunda, Bufo viridis* and *Hyla arborea*. The first three species constitute the guild of "Brown Anura" associated with forest and have the same spawning mode: females deposit a single clutch per year. The other three species prefer open areas and have multiple spawns. Brown Anura have larger eggs, and probably lower fecundity, than other amphibians of similar body size. Within the Brown Anura guild, different species show the same relationships of fecundity, egg size and clutch size with body length. Within the guild, lower fecundity and smaller eggs in species with smaller body size are compensated by rapid maturation. Among guilds, egg size is inversely correlated with fecundity.

INTRODUCTION

The reproductive success of amphibians is critically related to their pattern of spawning. Important variables include fecundity, egg size and total reproductive costs. Number of eggs per clutch (here and further: fecundity) relates to reproductive success in terms of the number of offspring, egg size in terms of their quality, reflected in the survivorship of larvae. Both developmental rates and hatchling size may depend on egg size (KAPLAN & COOPER, 1984, WALLS & ALTIG, 1986; WOODWARD, 1987; ISHCHENKO, 1989; but see WALLS & ALTIG, 1986, KAPLAN, 1987; WILLIAMSON & BULL, 1989). Perhaps an increase of reproductive effort may result in a decrease in the survival rates of females, as in other animal groups (PARTRIDGE & HARVEY, 1985).

For the clutch characters, three alternative ways of adaptation might be favoured. an increase in egg number per clutch, an increase in egg size or a decrease in total reproductive effort (if it tends to increase survivorship of adult females). Which way is favoured will depend on environmental conditions and specific morphology.

GILLER (1984) proposed to divide biological communities in associations including all coexisting species of the same taxon. Coexisting populations of related species often exploit similar resources and adapt to the environment in a similar way. In such a case they might be unified in guilds (ROOT, 1967). Any association may include one or more guilds.

Still now, no unified methods of dividing communities into guilds exist and intuition plays an important role (SIMBERLOFF & DAYAN, 1991). If an area is composed of two or more types of biota (e g. forest and grassland plots, etc.), the simplest way of outlining guilds is to unify all species attached to the same type of biota. Spatial preferences could be a good index of similarity in exploitation of the main resources (food, breeding sites, etc.).

Perhaps species of the same guild are similar not only in utilization of resources, but also in components of their life cycles, e.g. spawning mode. Connection between guild structure of associations and divergence of life cycles in coexisting species seems to be a poorly researched aspect of community ecology.

Six anuran species spread in Georgia appear to be a good example for investigation of this problem. They are sympatric in many localities. Nevertheless, they could be divided in two different guilds, according to their biotope preferences. Species of the first guild are strictly attached to forest during the terrestrial stage of their life. They form isolated, fully sympatric populations in the small mountain canyons. Species of the second guild prefer open places even in localities where wooded areas predominate. They form large populations in the river valleys, penetrating partly into the habitats of the first guild. The first guild includes *Rana macrocnemis*, *Bufo [bufo] verrucosissimus* and *Pelodytes caucasicus*. These species will be named here "Brown Anura" in accordance with their coloration, independent of their taxonomic position. The second guild includes *Rana ridibunda*, *Bufo viridis* and *Hyla arborea*, named here "Green Anura". A general description of the ecology of these species (except *B. viridis)* in Caucasus was given by TUNIYEV & BEREGOVAYA (1986).

The aim of the present investigation is the comparative analysis of spawning modes of these species. The study addresses the questions of which parameters of spawning may be common to species of the same guild but differ between guilds, and which parameters are variable within a guild

MATERIALS AND METHODS

The investigation was conducted in the Borjomi Canyon, Central Georgia. I studied amphibian habitats in the canyon of the river Nedzura (the right tributary of the river Kura). The stretch of the canyon from the mouth of Nedzura (where a village is located) to sources of the river exceeds 15 km (fig. 1). Elevation ranges from 900 to 1200 m. Annual precipitation amounts to 1000 mm. The river is framed by slopes covered with mixed forest (*Abies nordmanianna*, *Picea orientalis*, *Fagus orientalis*, *Carpinus caucasica*). The total area of terrestrial habitats in the canyon reaches about 460 hectares.

About 100 rain pools and pool sites along creeks, ranging from 1 to 3-4 thousands liters, are used as anuran spawning sites. Size, temperature, lighting and flowing regime of the different water bodies vary considerably.

Observations were made during April-July 1989-1991. All water bodies were inspected on every second to tenth day. Numbers of deposited clutches of each anuran species were

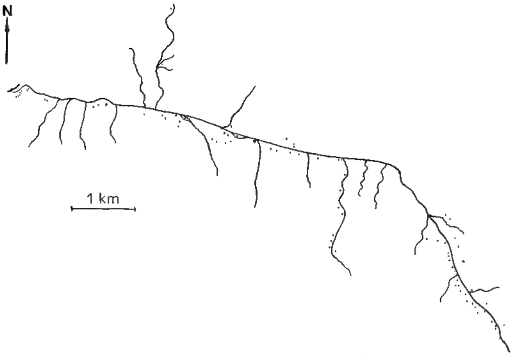

Fig. 1. Investigated part of the river Nedzura canyon (from the village to sources). Dots reflect the distribution of breeding sites

counted. The volume of each water body was estimated with the half-ellipsoid volume equation $v = \pi$ abc 6^{-1} . Tadpole number in all water bodies was estimated just before metamorphosis, at stages 40-41 (Gosner, 1960). This number was used as approximate value of metamorphosed offspring In some pools, the number of tadpoles was estimated visually. In other cases, up to 1000 tadpoles were stained in Neutral Red solution (Guttman & Creasey, 1973), released and recaptured 8-24 hours later. The total number of tadpoles was estimated with the Petersen method (Caughley, 1977) The number of pools where eggs or larvae were eliminated before metamorphosis was also counted.

Nineteen amplexed couples of *R. macrocnemis*, 21 of *P. caucasicus* and 29 of *B. verrucosissimus* were taken from spawning sites. Each couple was placed in a separate plastic box containing 3 l of stream water. After spawning, the following measures were taken: (1) body length (L = snout-urostyle length) of each female was measured to the nearest 0 1 mm, using callipers; (2) average diameter of 30 eggs in each spawn clutch (D) (for this aim, the outer envelope was removed from each egg before the first cleavage; eggs were transferred to petri dishes containing water; egg cell diameter was measured to an accuracy of 0.05 mm using a binocular with an eyepiece micrometer); (3) fecundity (N = number of eggs per clutch); in the small clutches of *P caucasicus* eggs were counted directly, while for the large clutches of *R. macrocnemis* and *B. verrucosissimus* the

following procedure was applied: a portion of each clutch containing 200 eggs with their envelopes was removed and its volume was measured; the total volume of the clutch was also measured immediately and, thus, total egg number was calculated; ten females of each species were killed after spawning for further examination of their ovaries.

Eighty-four naturally deposited clutches of *R. macrocnemis*, ten clutches each of *H. arborea* and *B. viridis*, and five clutches of *R. ridibunda* were also studied. In these clutches, D was measured at stage 9 (GOSNER, 1960). Five females of *B. viridis* and *R. ridibunda* and three females of *H. arborea* were caught just after spawning, killed, and their ovaries were inspected.

Egg volume (mm^3), calculated as $v = D^3 \pi 6^{-1}$, was used instead of D in the basic calculations. The total volume of clutch V (ml) was calculated as Nv. For estimation of specific volume of clutch, in comparison with body size of female, I used the index $SV = VL^{-3}$.

Standard methods of correlation and regression analysis (ZAITSEV, 1984) were used for establishing connections between separate measures. For each data sample, the arithmetic mean M and the main statistical parameters were estimated. For each compared pair of samples, the correlation coefficient R was calculated. When interdependence between two groups of data could be described as linear, parameters of a linear regression $y = ax + b$ were estimated. When interdependence was clearly curvilinear, parameters of the allometric equation $y = ax^b$ or the hyperbolic equation $y = a - bx^{-1}$ were estimated. Electivity of females to pools of different volumes was quantified using the electivity index of IVLEV (1961): $J = (P_i - P_i^*) (P_i + P_i^*)$, where P_i^* is the percentage of water bodies of the given class in the environment and P_i is the percentage of spawn clutches deposited in ponds of this class.

RESULTS

GENERAL DESCRIPTION OF SPAWNING MODES

Brown Anura breed in small water bodies in the forest area. *Rana macrocnemis* begins to spawn in early April, and the reproductive period lasts about two weeks. Females spawn in stagnant or seepage pools, seldom in slowly running creeks. Spawn is deposited as large clumps floating in water. *Pelodytes caucasicus* begins to spawn in May or early June, and the breeding period continues through October. It spawns in slowly running water bodies, rarely in stagnant pools. Spawn clumps are attached to aquatic vegetation or sunken objects. Larvae usually hibernate and metamorphose in the second year of their life *Bufo verrucosissimus* begins to breed in April; the breeding period continues through June It usually spawns in slowly running water bodies. Sometimes males wait for females in water as in other species, but more often mating takes place on land. Amplexing couples remain on the ground surface for up to one week, looking for appropriate breeding sites. If a satisfactory site is not found, females may spawn on the ground surface. Spawn is deposited in long cords. There were no visible oocytes in the ovaries of females of Brown Anura after spawning each female deposits a single clutch per year.

The main breeding sites of Green Anura are situated outside of the studied area, in the valley of river Kura. They are permanent ponds, often with rich vegetation. In the study area, Green Anura deposit spawn in warm rain pools. Breeding periods of all three species continue from mid-April to mid-July. *Bufo viridis* is the most undiscriminating in terms of breeding site selection. *Rana ridibunda* spawns only in the largest pools (500 l and more). Oocytes of different size were present in the ovaries of females of Green Anura having just spawned (except three females of *B. viridis*): probably each female deposits some egg portions during the breeding season. Mating occurs in water in all three species.

Body length and fecundity

The body length (L) of breeding females is shown in Table I. It increases in the order *P. caucasicus* - *R. macrocnemis* - *B. verrucosissimus* in the Brown Anura guild. Average L of *B. verrucosissimus* exceeds body length of *R. macrocnemis* by a factor of 1.6, and the latter exceeds body length of *P. caucasicus* also by a factor of 1.6. Number of eggs per clutch (N) increases between species with body size: *R. macrocnemis* deposits 3.1 times more eggs on average than *P. caucasicus*, and *B. verrucosissimus* 4.1 times more than *R. macrocnemis* (Table I).

Fecundity also depends on body size within populations. N correlates with L in *R. macrocnemis* and *P. caucasicus*. The highest value of the correlation coefficient R_{LN} was found in *R. macrocnemis* perhaps as a result of the high variability in female body length: $R_{LN} = 0.709$ ($n = 19$, $P < 0.001$; $N = 38.5\ L - 1267$). In *P. caucasicus*, the correlation coefficient was: $R_{LN} = 0.678$ ($n = 17$, $P < 0.001$; $N = 52.9\ L - 1835$). Correlation of N and L in *B. verrucosissimus* was not significant : $R_{LN} = 0.232$, $n = 29$.

To test the assumption of a common allometric relationship, I estimated the correlation between the logarithms of N and L for a combined sample, including data of all three species. Correlation of logarithmic data was 0.962 ($n = 65$, $P < 0.0001$; $N = 0.0136\ L^{2.73}$) (fig. 2).

Body size and egg size

Species of the Brown Anura guild with larger body size have larger eggs (Table I). Moreover, correlations between L and D were found within populations. Correlation coefficients in *P. caucasicus* and *R. macrocnemis* populations were 0.669 and 0.800, respectively. Correlation coefficients of v and L were 0.684 and 0.704 (n respectively 17 and 19, $P < 0.001$ in both cases; in *P. caucasicus*, $v = 0.137\ L - 3.62$; in *R. macrocnemis*, $v = 0.132\ L - 4.60$). In the *B. verrucosissimus* population, egg size was not correlated with female body size: $R_{LV} = 0.121$.

If data for the three species are lumped together, a clear curvilinear connection between v and L appears. Correlation of logarithmic data is 0.772 ($n = 65$, $P < 0.001$). Empirically this connection could be satisfactorily described by the hyperbolic equation $v = 7.9 - 230.5\ L^{-1}$ (fig. 3).

Table I. - Reproductive characteristics of anurans inhabiting Borjomi Canyon. L: body length (mm); N: number of eggs in the clutch; D: diameter of fertilized eggs (mm); v: volume of eggs (mm^3); SV: specific volume of clutch, $SV = VL^{-1}$, where $V = vN$; n: sample size; M: arithmetic mean; S: standard deviation; SE: standard error; CV: variation coefficient (%). Pc: *Pelodytes caucasicus*; Rm: *Rana macrocnemis*; Bve: *Bufo verrucosissimus*; Ha: *Hyla arborea*; Rr: *Rana ridibunda*; Bvi: *Bufo viridis*.

		n	M	S	SE	CV	Limits
Pc		21	44.64	2.13	0.46	4.8	40.5-48 0
Rm		19	71.50	6.62	1.52	9.3	53.3-79.0
Bve		29	113.74	4.85	0.90	4.3	102.6-123.5
Ha	L	5	51.9	0.63	0.28	1.2	51.3-53.1
Rr		20	98.8	2.63	0.59	2.7	92.7-105.2
Bvi		16	81.3	7.94	1.99	9.8	51.9-93.8
Pc		21	493	147	32	29.8	100-750
Rm	N	19	1513	377	87	24.9	750-2100
Bve		29	6145	1815	337	29.5	3100-10000
Pc		17	1.69	0.13	0.03	7.6	1.4-2.1
Rm		19	2.11	0.19	0 04	9 1	1.7-2 4
Bve		29	2.22	0.15	0 03	6 9	1 9-2 5
Ha	D	10	1.33	0.12	0.03	9.0	1.1-1.6
Rr		5	1.72	0.05	0.02	2.9	1.6-1.9
Bvi		10	1.36	0.10	0.02	7.2	1.1-1.6
Pc		17	0.0141	0.0040	0.0009	28.6	0.004-0.021
Rm	SV	18	0.0184	0.0047	0.0012	25.7	0.010-0.032
Bve		29	0.0241	0.0097	0.0032	40.4	0.012-0.046

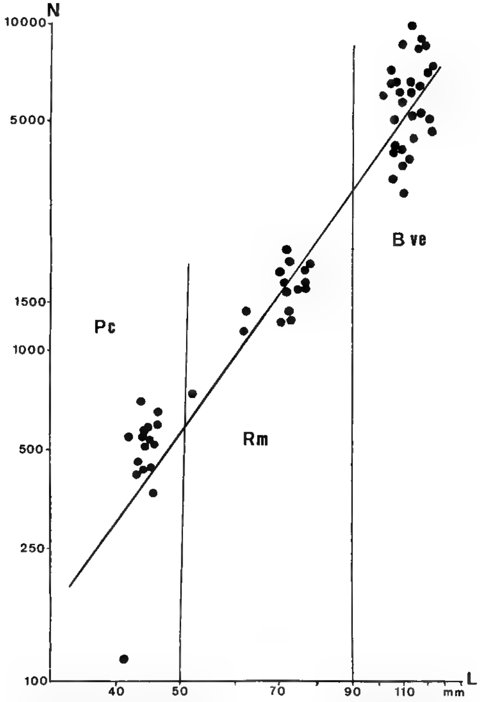

Fig. 2. — Correlation of egg number in clutch and body length of female (only for Brown Anura guild; logarithmic scale). N: egg number; L: body length (mm). Solid line reflects regression of N on L: $N = 0.0136\ L^{2.732}$. Bve: *Bufo verrucosissimus*; Pc: *Pelodytes caucasicus*; Rm: *Rana macrocnemis*.

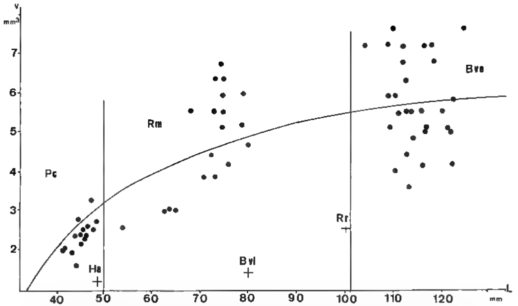

Fig 3. — Correlation of arithmetic mean of egg size in clutch and body length of female. v: mean egg volume (mm³), L: body length (mm). $v = 7.9 - 230.5\ L^{-1}$. For Green Anura, only arithmetic means of values are shown. Bve: *Bufo verrucosissimus*; Bvi: *Bufo viridis*; Ha: *Hyla arborea*; Pc: *Pelodytes caucasicus*; Rm: *Rana macrocnemis*; Rr: *Rana ridibunda*.

Eggs of Green Anura are smaller than eggs of Brown Anura for animals with similar body size (fig. 3). Egg size is related to female body size as in the Brown Anura guild. *Hyla arborea* has the smallest eggs, *R. ridibunda* the largest ones (Table I, fig. 3).

EGG SIZE AND FECUNDITY

No correlation between v and N was found within separate Brown Anura populations. For 84 naturally deposited *R. macrocnemis* clutches, correlation between v and N was 0.167, $P > 0.05$. Linear correlation between N and v exists at the across-species level ($R_{vN} = 0.947$, $P < 0.001$, $n = 84$, $v = 0.661\ N - 0.027$). Common dependence of N and v on L provides the basis of this connection.

BODY SIZE OF FEMALE AND VOLUME OF CLUTCH

We can judge variability in volume of clutches only for Brown Anura. In *P. caucasicus* and *R. macrocnemis* populations, a positive correlation between L and V exists (0.862 and 0.828 respectively, $n = 17$ and 19, $P < 0.001$). Coefficients of linear regressions of V on L are 0.218 and 0.345 (fig. 4). In *B. verrucosissimus*, correlation of L and V is absent, R_{LV}

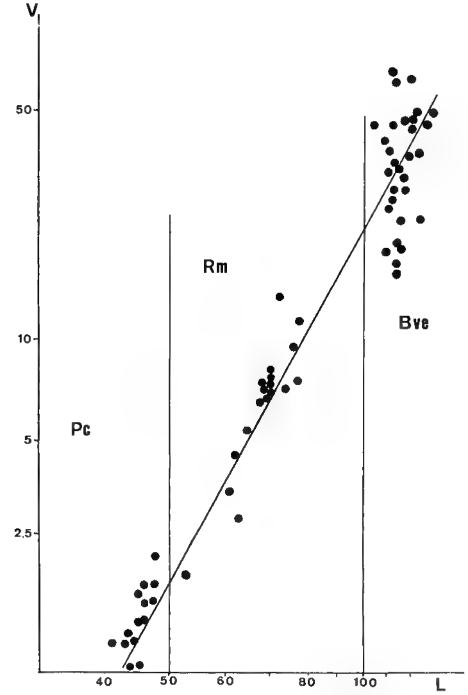

Fig. 4. — Correlation of body length and clutch volume in Brown Anura guild (logarithmic scale). V = vN - clutch volume (ml), L: female body length (mm). $V = 0.00000171\ L^{3.55}$. Bve: *Bufo verrucosissimus*; Pc: *Pelodytes caucasicus*; Rm: *Rana macrocnemis*.

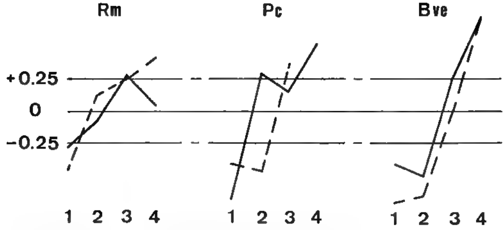

Fig 5. — Electivity of spawning sites of different sizes. Continuous lines, 1989, dashed lines: 1990 (1): water bodies of volume to 20 l; (2): to 160 l, (3) to 1280 l; (4) more than 1280 l Bve: *Bufo verrucosissimus*, Pc: *Pelodytes caucasicus*; Rm: *Rana macrocnemis*

— 0 123 Lumping together data of all three species, a curvilinear relation between L and V was obtained. Correlation of logarithmic values was 0.972 ($n = 64$, $P < 0.001$). The relation $V = 0.0000017\ L^{3.6}$ (fig. 4) shows that clutch volume increases stronger than female body volume. The allometric relation of N and L is strengthened by a positive correlation between body size and egg size. For all three species taken together, there is also a positive correlation between SV and L: $R_{L,\ SV} = 0.501$, $n = 64$, $P < 0.001$, SV = 0,141 L - 8,66.

EXPLOITATION OF BREEDING SITES AND SPATIAL STRUCTURE OF ANURAN POPULATIONS

The number of available breeding sites appears to be the main factor limiting population numbers of anuran species. During 1989-1990, spawn was deposited in 188 small water bodies in the study area. The total number of clutches of Brown Anura species is presented in Table II. Most of the pools dried up or were washed off by rains before metamorphosis was completed. A few water bodies yielded emergence of most offspring (Table II). All Brown Anura species avoided the smallest water bodies and preferred the largest ones (fig. 5). As a result, spawning often took place in pools already populated with larvae of other species (Table III). In such cases, growth of younger tadpoles was retarded. Nevertheless, after metamorphosis of older larvae, growth and development of younger tadpoles renewed and they passed metamorphosis. As a result, even limited numbers of effective breeding sites in the canyon ensure quite high generation numbers in all Brown Anura populations.

The situation in Green Anura species is different. In the Nedzura canyon, their reproductive niches are included fully in the reproductive niche of *R. macrocnemis*. Large

Table II. - Egg and larval mortality in Brown Anura populations. 1: number of water bodies where spawning took place; 2: total number of clutches deposited in the canyon; 3: number of breeding sites where metamorphosis was completed; 4: number of clutches taking part in the formation of the new generation; 5: approximate number of tadpoles surviving to GOSNER stages 40-41 in all breeding sites of the canyon; 6: number of tadpoles in breeding sites with lowest mortality rates (the number of such water bodies is given in brackets); 7: survival rates from egg to metamorphosis in all sites (%); 8: survival rates in most effective breeding sites (%). Pc: *Pelodytes caucasicus*; Rm: *Rana macrocnemis*; Bve: *Bufo verrucosissimus*.

	1989			1990		
	Pc	Rm	Bve	Pc	Rm	Bve
1	46	56	17	28	89	17
2	410	362	49	331	1037	86
3	10	13	5	13	10	8
4	150	172	14	260	235	72
5	6000	15500	3500	6000	44500	45000
6	4000(1)	13073(2)	3000(3)	4000(1)	35703(3)	40000(1)
7	4.92	0.83	1.16	6.10	0.92	8.52
8	14.96	11.34	5.42	14.33.	19.51	12.52

Table III. - Overlap of breeding sites explored by different Brown Anura species: the percentage of water bodies already populated with larvae of species B from all water bodies where spawning of species A took place. Bve: *Bufo verrucosissimus*; Pc: *Pelodytes caucasicus*; Rm: *Rana macrocnemis*.

A	B	1989	1990
Rm	Pc	27.3	13.7
Pc	Rm	16.7	75.2
Pc	Bve	17.4	15.5
Pc	Rm, Bve	21.5	84.8
Bve	Rm	14.9	18.9
Bve	Pc	53.2	17.2
Bve	Rm, Pc	65.9	23.1

tadpoles of this early-breeding species were present in almost all spawning sites of Green Anura. As a result, larvae of these species were usually eliminated already in early developmental stages. Emergence occurred only in pools which were not populated with Brown Anura larvae. Only a few offspring of Green Anura emerged during the year in the study area: some hundreds *B. viridis*, some tens *H. arborea* and a few specimens of *R ridibunda*. This amount is not enough for renewal of Green Anura neighborhoods populating the canyon. These neighborhoods depend on immigrants from the river Kura canyon, where large permanent ponds are present and breeding is more efficient.

DISCUSSION

In boreal associations of Anura, species attached to wooded areas (brown frogs, *Bufo bufo* complex, etc.) often coexist with species preferring open plots (*Rana* kl. *esculenta* complex, green toad, etc.) (e. g. TUNIYEV & BEREGOVAYA, 1986; PIKULIK, 1985; etc.) Green frogs and green toads are most widely distributed, penetrating even into urbanized territories. "Forest" species are commonly early-breeders (BANNIKOV et al., 1977). In localities where forest areas predominate, they have an advantage in utilization of spawning sites due to well known priority effects (HEUSSER, 1972; PIKULIK, 1976; ALFORD & WILBUR, 1985; etc.). For example, *Rana sylvatica* tadpoles suppress *Hyla crucifer* tadpoles when they breed in the same ponds (MORIN & JOHNSON, 1988); *Rana temporaria* and *Bufo bufo* suppress development of *Bufo calamita* (HEUSSER, 1972; BANKS & BEEBEE, 1987; GRIFFITHS, 1991). In the association described here, "Green Anura" are weak competitors in small temporary pools, though species of this guild have wider possibilities of exploitation of large permanent ponds, most of which are situated in open areas.

On the other hand, hot and dry main terrestrial habitats of Green Anura are perhaps less favourable than humid forest habitats. As a result, Green Anura appear to be stronger competitors in the terrestrial stage of life whilst Brown Anura are stronger competitors during larval development.

The common spawning strategy of Green Anura species includes: diminishing of reproductive costs due to unfavourable terrestrial habitats; perhaps increasing fecundity in more predictable spawning conditions. Diminishing of reproductive costs is attained via asynchronous development of oocytes and portional spawning. Portional spawning is known for many species of Anura distributed in countries with a hot climate (see DUELLMAN & TRUEB, 1986, for review). Interestingly, *R. ridibunda* and *B. viridis* in northern parts of their range deposit a single clutch during the breeding season (e. g. KUBANTSEV et al., 1979, PIKULIK, 1985; GOROVAYA & DZHANDAROV, 1986) Increasing fecundity is attained at the expense of egg size. I have no information about the actual fecundity of Green Anura in the studied area, but fecundity of *H. arborea*, *R. ridibunda* and *B. viridis* was investigated in different regions by previous authors. Individual *H. arborea* females deposit 744.8 $\pm$ 45.5 eggs in Czechoslovakia (MORAVEC, 1989) and up to 1000 eggs in Ukraine (SCZERBAK & SCZERBAN, 1980). *Rana ridibunda* females deposit 4000-12000 eggs in different parts of this species' range (AVRAMOVA et al., 1976; KUBANTSEV et al., 1979; GOROVAYA & DZHANDAROV, 1986). A single full clutch of *B.*

viridis found in our study contained about 10000 eggs. In different regions, *B. viridis* females deposit 6000-14500 eggs (AVRAMOVA et al., 1976; KUBANTSEV et al., 1979). These values are significantly higher than those obtained for Brown Anura of comparable body size. Fecundity of brown frogs and common toads in Europe is also lower than estimates cited for *R. ridibunda* and *B. viridis* (GIBBONS & MCCARTHY, 1986; READING, 1986).

Allometric interdependence between N and L reflects the fact that N increases with body volume, not body length: $N = aL^b$, where $b \approx 3$. When $b < 3$, oocyte number increases slower than body volume; when $b > 3$, oocyte number increases faster than body volume. Interestingly, the general allometric index of the relation of N and L obtained for Brown Anura (2.73) is equal to an analogous value established for the grass frog *Rana temporaria* (GIBBONS & MCCARTHY, 1986). This species is ecologically rather similar to *R. macrocnemis*. Inversely, in the rice frog *"Rana" limnocharis* (characterized by portional spawning), this index is 3.47 (SHICHI et al., 1980).

Species spawning in small temporary pools have larger eggs and lower fecundity than related species which spawn in permanent water bodies (CRUMP, 1981, 1989; WOODWARD, 1987; RAFINSKA, 1991). Accordingly, eggs in Brown Anura species are larger than in Green Anura of comparable body size. Egg size increases with female body size more rapidly in this guild (fig. 3). Increase of egg size as well as increase of egg number per clutch are limited by female body size in both guilds. But among Green Anura, fecundity increases at the expense of egg size; in Brown Anura, egg size increases at the expense of fecundity.

An additional factor determining the equilibrium point between egg size and fecundity appears to be environmental temperature. Increase of temperature causes conservation of high fecundity at the expense of egg size in *Bombina orientalis* (KAPLAN, 1987). This dependence may be generalized to the interspecific level. Temperature in the main habitats of Green Anura species is higher than in the Brown Anura habitats.

Within Brown Anura, differences in spawning mode are connected with female body size. The choice of the way of adaptation is whether rapid maturation but a small clutch will be favoured or a large clutch but postponement of maturation. Smaller Brown Anura species mature in shorter time than larger ones (GOKHELASHVILI & TARKHNISHVILI, in prep.). Rapid maturation decreases the period between generations and increases the number of adult animals. On the other hand, body size remains small and fecundity low. Egg size is also limited. Larger Brown Anura species increase their reproductive effort at the expense of the period between generations.

The taxonomic position of a species is not connected with its position neither on the "macrohabitat" nor on the "body size" axes. Both pairs of congeneric species (*Rana* and *Bufo*) are divided among two different guilds.

BEGON et al.(1986) noted that interspecific differences of life cycles often deviate from the framework of the widely known r-K model. Perhaps the direction of the life cycle variation between any two species depends on their position in the guild structure of the community. For our case, species of the same guild vary both in fecundity and egg size but have a common relation between these characters and female body size. Species of different guilds have a different correlation between egg size, fecundity and female body

size. The main reason of interspecific differences within a guild appears to be species-specific body size, whereas between guilds environmental conditions in the main terrestrial habitats seem to be important

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Summertime population density of *Rana temporaria* in a Finnish coniferous forest

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In this study, two methods were used to determine the distribution and the density of the frog population in a dry coniferous forest near a site where thousands of frogs winter in the ponds of a gravel pit. The fence-with-traps method gives relative values (frogs/100 m/day) and the square method gives the number of frogs per area. These methods are suitable only for mature and large immature frogs because small immature frogs (snout to urostyle < 35 mm) were able to climb up along the walls of plastic buckets and fences used for trapping.

With the square method, 64-80 mature frogs/ha were captured in 1990. The fence-with-traps method showed that the population density was highest close to the wintering site (2.3 frogs/100 m/day at a distance of 50 metres) and decreased with the distance from it (0.17 frogs/100 m/day at a distance of 500 metres). This decrease of density was caused by lower numbers of immature frogs.

INTRODUCTION

A wintering and spawning site of the common frog (*Rana temporaria*, Ranidae) is located in an old gravel pit in Laikko, Rautjärvi, Finland (61°22'N, 29°17'E), as has also been mentioned in previous reports published in Finnish (PASANEN et al., 1989, 1990). The frog winters in Finland almost only in water environment (KOSKELA & PASANEN, 1974). The habitat of the common frog during its feeding period has been described as a meadow with luxuriant vegetation composed of tall herbs and grasses (e.g. LOMAN, 1976, 1981, 1984). In Laikko, however, dry coniferous forest surrounds the ponds of the gravel pit where thousands of frogs winter and spawn The nearest potential wintering and spawning sites are the lakes 700 metres from the gravel pit.

LOMAN (1976, 1981, 1984) has used the capture-recapture method for studying the densities of frog populations. The fence-with-traps method has been used to count migrating frogs in spring or in autumn (KOSKELA & PASANEN, 1974; PASANEN et al., 1989, 1990). The main material for this study was gathered using the fence-with-trap method. This method gives values of relative population density (e.g frogs/100 m/day). The aim of this study was to determine population density at different distances from the wintering site. In 1990 also the square method was used to determine population density as frogs/ha.

Table I. — Distances of the fences and of the squares from the wintering site of *Rana temporaria*, lengths of the fences and numbers of trapping days in 1989 and 1990.

Fence	Distance	Length	Days		Square	Distance	Days
			1989	1990			1990
I	50 m	250 m	49	30	A	100 m	42
II	250 m	200 m	32	23	B	200 m	40
III	450 m	400 m	40	0	C	300 m	40
IV	500 m	400 m	25	0	K	120 m	34

Material and methods

In the study area, frogs spawn usually at the end of April or in the beginning of May, and the migration to the wintering site takes place in September and October. In the spring of 1989, when they started to move from the wintering and spawning pond, 2042 (977 mature and 1065 immature) frogs were marked collectively by toe-clipping. In 1989, four fences-with-traps (Koskela & Pasanen, 1974) were built to study the dispersion of frogs from the gravel pit (fig. 1, Table I). The fence was made of a 30 cm high plastic sheet stretched vertically between wooden sticks without any horizontal part on the top. The lower edge of the sheet was covered by moss to provide a tight seal against the ground. Buckets (diameter 25 cm, depth 22 cm, angle 95°) were buried into the soil at 10 m intervals along the fence which crossed in the middle of the buckets, so that frogs from both sides were able to fall into the buckets

The total trapping period in 1989 lasted from the end of May to the beginning of August. The buckets were examined and emptied almost every other day. In 1990, only fences I and II were used during June-July.

All frogs caught in the buckets during summer were measured (from the snout to the end of the urostyle). The material was grouped as follows: immature specimens (length smaller than 64 mm) and mature individuals (length greater than 64 mm) according to Koskela & Pasanen (1974). The frogs were marked collectively by toe-clipping (the same toe for all summer captures, but not the same toe as in the spring) and the adults were also sexed The frogs were then set free 2-3 metres from the pitfall haphazardly on either side of the fence. The results are given as number of frogs per 100 metres of fence in 24 hours (frogs/100 metres/day).

In June 1989, seven 10 × 10 metres squares were built at the distance of 350-400 metres from the wintering pond between the fences II and III (fig. 1). The fence was again 30 centimetres high and every square had one bucket in each corner.

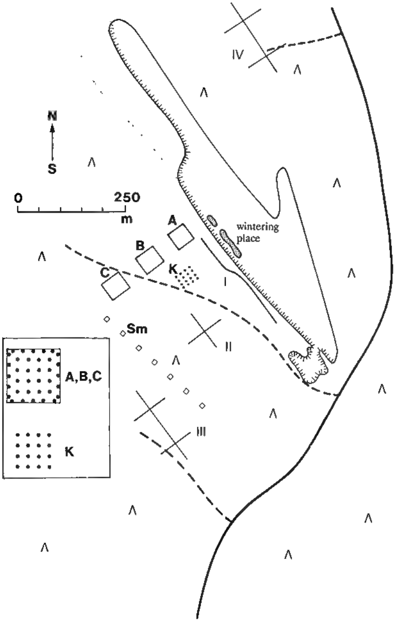
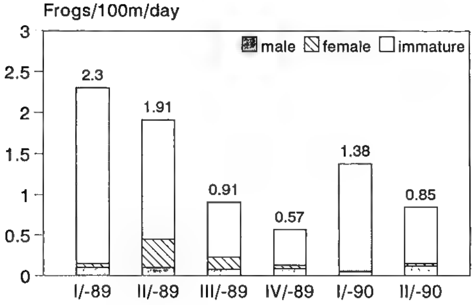


Fig. 1. — Map of the study area.
I-IV = fences-with-traps (buckets placed at 10 m intervals).
A-C = fenced squares (50 × 50 m, 36 buckets)*.
K – square without fence (16 buckets)*.
Sm = small squares (10 × 10 m).

* The small inset figure shows the location of buckets in squares A-C and K.



	I/-89	II/-89	III/-89	IV/-89	I/-90	II/-90
immature	2.15	1.46	0.68	0.44	1.32	0.7
female	0.05	0.35	0.15	0.04	0.01	0.03
male	0.1	0.1	0.08	0.09	0.05	0.12

Fig. 2. – Frogs caught with the fence-with-traps method in 1989 and 1990 on fences I-IV (see fig. 1).

In the beginning of June 1990, three 50 × 50 metres enclosures (A-C) were built to count natural frog density (fig. 1, Table I). The plastic fence was 50 centimetres high and the lower edge of the fence was buried 10 centimetres into the mineral soil. In each square, 20 buckets were buried along the fence (inside), and the inner part of the square was furnished with 16 buckets placed at a 10 m distance from each other. The control plot consisted of 16 buckets without a fence (square K) (see fig. 1).

Frogs were trapped in squares and at fences I and II from June 2 to August 10, 1990. The buckets were checked and emptied and the frogs were treated as in the previous summer. During autumn (September 18 – November 3, 1990), frogs began to gather into the side buckets of the squares on their way to the wintering sites. These frogs were counted and released outside the square. A total of 36 recently metamorphosed frogs were marked to control their possible crossing of a fence.

Weather conditions (temperature and humidity) were recorded with a Lambrecht-hydrothermograph during both summers. Mean daily temperature and mean daily relative humidity were calculated as the mean of four observations (00.00, 08.00, 16.00, 24.00 h) of the day before the catching day.

Table II. — Proportion of adults among frogs marked in the spring 1989 (977 mature, 1065 immature) and caught during the summer at fences I-IV.

Fence number	Recaptured (mature/immature)	Percentage of adult marked frogs
I	8/60	13%
II	6/23	25%
III	16/20	81%
IV	6/8	83%

RESULTS

The total number of frogs caught in the summer of 1989 was 555 (85 mature and 470 immature). The number of immature frogs decreased significantly (Pearson's correlation, $R^2 = 12.36$, $P < 0.01$) as the distance from the wintering site increased (fig. 2). Most females were caught at fences II and III, while the number of male frogs was nearly the same at all four fences. In 1990, the actual number of captured frogs was 133 (10 mature and 123 immature).

The proportion of adult frogs marked in the spring of 1989 at the wintering site increased with increasing distance from the gravel pit (Table II). Of the 555 frogs caught and marked at fences I-IV in the summer of 1989, only 29 individuals (5 %) were recaptured in the same summer and none in 1990. Recaptures were more common among adults (14 %) than among immature frogs (3 %).

In the small squares (10 × 10 metres), 0-2 mature frogs were found per square (mean = 1.29, S.E. = 0.29, n = 7), which would make 129 frogs/hectare. In the large (A-C) squares, 10-17 frogs/square were caught in summer and 16-20 in autumn (Table III). All frogs marked inside the squares during summer and three to six unmarked frogs/square were found in the autumn. They did not fall into the traps during summer, even though the trapping period lasted 40-42 days The total number of mature frogs/ha varied from 64 to 80.

Small immature frogs (juveniles in their first year, snout to urostyle < 35 mm) were able to climb up along the walls of the plastic buckets and fences. This was indicated by the marked frogs and the increase in the number of immature frogs caught during summer. Eight (24 %) of the 36 small frogs marked in autumn and released outside the squares were found again inside a square later on. In the summer (at the beginning of metamorphosis), only 3-11 small frogs were found in the squares (12-44 immature/ha), but, during the autumn trapping, 140-190 small, recently metamorphosed frogs were captured per square (560-760 immature/ha) Although this method proved to be unsuitable for density

Table III. — Number of mature frogs in the squares.
Summer = catches in summer (including buckets along the fence and in the inner part of the square).
Autumn = catches in autumn.
Inner buckets = catches in the buckets in the inner part of the square in summer (number of catching days in parenthesis).

Square	Summer				Autumn			
	♂	♀	Σ	Inner buckets	♂	♀	Σ	Frogs/ha
A	13	4	17	6 (42)	15	5	20	80
B	10	4	14	8 (40)	12	7	19	76
C	10	0	10	5 (40)	13	3	16	64
K	1	0	1	1 (34)	-	-	-	-

estimation of the smallest frogs, it shows that in the autumn the immature frogs are abundant at a distance of hundreds of meters from their home pond.

No correlation was found between the activity of the frogs and weather conditions. Temperature apparently did not influence the activity of frogs during summer (1989 R^2 = 1.53, P > 0.60; 1990 R^2 = 1.76, P > 0.60), nor did relative humidity (1989 R^2 = 4.20, P > 0.40; 1990 R^2 – 10 94, P < 0.25).

Discussion

The fence with traps method is generally used to study frog migrations in the spring or autumn. In this study, we tried to use the method also to study the distribution and population structure. The method gives only relative results, but when several fences are used at the same time, the results are comparable. In this study we got clear results: the number of immature frogs decreases as distance from the wintering site increases, but equal numbers of mature frogs were found at different distances (fig. 2).

We used the square method in order to estimate the population density. These results (64-80 mature frogs/ha) agree with those of other studies. On meadows, the population density of *Rana temporaria* can be as high as 550-790 mature frogs/ha (Loman, 1976, 1981, 1984). In deciduous forests, it has been estimated that there are 125 mature frogs/ha (Glowacinski & Witkowski, 1970), and in coniferous forests 25-50 adult frogs/ha (Inozemtsev, 1969). The method used in the cited studies was capture-recapture. In the present study, a density of 100 frogs/ha is reached, if large immature frogs are included

(frogs longer than 64 mm were considered to be adults). Since the ground vegetation in Laikko is very poor and includes in many areas only mosses, the population density seems to be rather high for these conditions. In 1989 and 1990, nearly 3000 mature frogs were found to winter in the ponds at Laikko (PASANEN et al., 1989, 1990). If the population density around the gravel pit in summer remained constant (64-80 frogs/ha), 3000 frogs would require an area with a radius of 350-400 m.

In areas around a spawning pond, favourable conditions during spawning and the larval phase have a strong impact on population density (SAVAGE, 1961, LOMAN, 1978; CUMMINS, 1986). In Laikko, the ponds in the gravel pit are the only spawning places of the area. According to the density results, the number of frogs, especially young ones, decreases as the distance from the ponds increases Of all the marked frogs, the proportion of mature frogs was greater as the distance from the wintering site increased. This indicates that the spawning and wintering site is the core of the population, from which immature frogs gradually move farther away.

This type of population is quite vulnerable Spawning failure in one spring is not as dramatic as is failure to winter Wintering may be unsuccessful due to draining of the pond, for example, and this can cause the population to collapse for a long time. Catches for the years 1989 and 1990 were different: in 1989, there were many more immature and female frogs than in 1990 (fig. 2). This raises the question: is it possible that frogs especially female and immature frogs could also winter on the ground, when the soil doesn't freeze deeply?

In square K were captured only one mature frog, while in the corresponding buckets of the inner part of squares A-C there were 5-8 frogs. Obviously the fence confuses the frogs, and a 50 × 50 m square is therefore too small for studying their movements. On the other hand, many frogs do not move enough during the whole summer to fall into a bucket (Table III).

The square method is good for estimating population density of the frogs. With 10 × 10 m squares, mere chance determined whether there were two frogs, one frog or none inside a square. Squares of 50 × 50 m, however, seemed to be suitable in this biotope. When population density is high, it may also be possible to obtain reliable results with smaller squares. We recommend to build squares in late summer and to do empty-catching during the autumn migration of the frogs, no buckets are needed in the inner part of the square. The observation that young frogs which have not yet wintered are able to climb over a plastic fence and up the sides of a bucket decreases the usefulness of these methods, which are suitable only for mature and larger immature (> 35 mm) frogs.

In many studies, correlations between temperature and humidity and the activity of frogs were observed (BELLIS, 1962, DOLE, 1965; ASHBY, 1969, LOMAN, 1979; WOOLBRIGHT, 1985). In this study, we could not find any correlation between weather conditions and movements of the frogs. A possible explanation is that the buckets were usually examined every second day and the long period between examinations covered the correlation. Thus the buckets ought to be examined at least once a day.

Résumé

Dans cette étude, deux méthodes ont été utilisées pour déterminer l'activité et et la densité d'une population de grenouilles dans une forêt de conifères assez sèche près d'un site où des milliers de grenouilles hivernent dans les étangs d'une gravière.

La méthode qui fait appel à des pièges placés le long d'une barrière donne des valeurs relatives (grenouilles/100 m/jour). La méthode des carrés donne le nombre des grenouilles par surface Ces méthodes ne conviennent que pour l'étude des grenouilles de taille importante, car les petits spécimens encore immatures (< 35 mm) sont capables de grimper le long des seaux en plastique et le long des barrières utilisées pour les piéger.

En 1990, avec la méthode des carrés, 64 à 80 grenouilles matures ont été piégées par hectare. La méthode des barrières a démontré que la densité de la population était la plus grande près du site d'hibernation (2,3 grenouilles/100 m/jour à une distance de 50 mètres), et que cette densité diminuait avec l'augmentation de cette distance (0,17 grenouilles/100 m/par jour à une distance de 500 mètres). La baisse de la densité totale est causée par une diminution de la densité des jeunes grenouilles quand la distance augmente.

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Workshop on population biology of amphibians

A workshop entitled "Population biology of amphibians" will be held in Vienna (Austria) from Wednesday 14th to Saturday 17th September 1994. Round table discussions on the topics "Demography of amphibian populations" and "Dispersal and gene flow among amphibian populations" will be the main events of the workshop. Poster presentations on all aspects of amphibian population biology will be welcome. The program will also include a few invited lectures and an excursion.

The workshop will be jointly organized by the Austrian Academy of Sciences, the International Society for the Study and Conservation of Amphibians (ISSCA) and the Österreichische Gesellschaft für Herpetologie (ÖGH, the Austrian Herpetological Society). Persons interested in participating should contact Dr. Walter Hödl or Dr. Günter Gollmann, both at the Institut für Zoologie der Universität Wien, Althanstraße 14, 1090 Wien, Austria (fax: (+431) 31336 700).

The 1994 General Meeting of ISSCA will be held in Vienna during this workshop (on Friday 16th September). For information, please contact Dr. Alain Dubois, ISSCA General Secretary, Laboratoire des Reptiles et Amphibiens, Muséum national d'Histoire naturelle, 25 rue Cuvier, 75005 Paris, France.

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